THE EFFECT OF X-IRRADIATION ON CHIASMA FREQUENCY IN *CHORTHIPPUS BRUNNEUS*

M. WESTERMAN*
Department of Genetics, University of Birmingham, England

Received 20.viii.70

1. Introduction

THE formation of chiasmata during or immediately prior to pachytene in eukaryotic cells can no longer be thought of as an isolated event. The weight of evidence to date suggests that it is the end-product of an interrelated series of processes initiated at some earlier stage of meiosis, some or all of which can be affected by a variety of physical and chemical agents. In the desert locust, *Schistocerca gregaria*, Westerman (1967) has shown that following exposure of males to a single dose of X-rays there are three discrete periods at which chiasma frequency may be altered. Thus irradiation during the S-phase of spermatogonial mitoses and during leptotene-early zygotene gave a significant increase in chiasma frequency when scored at diplotene-diakinesis. In contrast, an equal dose given during premeiotic DNA synthesis led to a significant decrease.

These alterations in mean cell chiasma frequency in *S. gregaria* appear to be the result of alterations in the number of chiasmata formed by the long and medium bivalents only, the three small bivalents continuing to form a single chiasma under all conditions. Since the chromosomes of this species, though all telocentric, do not respond in the same way, it seemed worth while extending the study to another grasshopper species, *Chorthippus brunneus* (Thunb.)—one which includes metacentric as well as telocentric elements in its complement.

2. Material and methods

In the first of two duplicate experiments, 87 fifth instar and young adult male *Chorthippus brunneus* from a laboratory culture of animals originating from the wild were divided into three groups. All individuals from two of these groups were injected abdominally at time zero with about 1·25 μCi of ^{3}H-thymidine in 0·03 ml. distilled water (Thymidine-6T$_{(n)}$ 5·00 Ci/mM R.C.C. Amersham). One of these two labelled groups was then immediately given a dose of 150 r. X-rays (for details see Westerman, *loc. cit.*). The experiment thus consisted of a control group, a labelled control group and an irradiated labelled group of insects. All three groups were subsequently maintained in an incubator at 30° C. and fed fresh grass daily. At appropriate time intervals (table 1, A) insects were taken at random from each group, their testes removed by vivisection and fixed in 1 : 3 acetic alcohol.

In the second experiment, 31 young adult males were injected with the same amount of ^{3}H-thymidine and irradiated with 150 r. X-rays as described above. These animals were then maintained in the same incubator at

* Present address: Department of Genetics, La Trobe University, Melbourne, Australia.

30° C. and individuals sacrificed at random at appropriate time intervals (table 1, B).

TABLE 1

The number of individuals sampled at each fixation time

		Fixation time in days post-injection																
	Experiment	1	2	3	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	Total
	Control	2	2	2	2	2	2	2	2	1	1	2	2	1	2	1	1	11	1	28
A	Control + H^3-Thymidine	2	2	1	2	2	2	2	2	1	1	2	2	1	1	1	1	0	0	25
	150r + H^3-Thymidine	2	2	2	2	2	2	2	2	1	2	2	2	1	2	2	2	2	2	34
B	150r + H^3-Thymidine	0	2	1	0	0	2	2	2	2	2	2	3	3	2	2	2	2	2	31

Prior to scoring, each individual was coded and randomised and chiasma frequencies were scored from lacto-propionic orcein stained squash preparations made from a part of each testis. Where possible, chiasma scores were recorded at diplotene-diakinesis for 20 cells per individual. In experiment A, three of the irradiated individuals, one from each of days 9½, 10 and 10½ post-irradiation, contained no suitable cells.

TABLE 2

Mean cell chiasma score $\pm \overline{sd}$ *for each sampling time shown in table 1*

Time in days	Treatment					
	Or	Or + H^3	Control	150 r. A + H^3	150 r. B + H^3	150 r.
+1	13·60 ± 0·1783	14·35 ± 0·1913	13·98 ± 0·1366	14·35 ± 0·1810	—	14·35 ± 0·1810
+2	13·95 ± 0·1992	13·40 ± 0·1747	13·68 ± 0·1352	14·38 ± 0·1114	14·97 ± 0·2337	14·65 ± 0·1280
+3	13·78 ± 0·2163	12·50 ± 0·1701	13·35 ± 0·1728	14·66 ± 0·1777	14·75 ± 0·1619	14·67 ± 0·1783
+4	13·40 ± 0·1518	13·23 ± 0·1409	13·31 ± 0·0951	13·70 ± 0·1527	—	13·70 ± 0·1527
+4½	13·75 ± 0·1670	13·68 ± 0·2160	13·71 ± 0·1357	13·45 ± 0·1289	—	13·45 ± 0·1289
+5	13·50 ± 0·1861	13·43 ± 0·1957	13·46 ± 0·1342	12·88 ± 0·2184	14·00 ± 0·1661	13·36 ± 0·1577
+5½	12·73 ± 0·1640	13·03 ± 0·1735	12·88 ± 0·1198	13·98 ± 0·1660	13·38 ± 0·1589	13·68 ± 0·1190
+6	14·60 ± 0·2449	13·25 ± 0·1670	13·70 ± 0·1600	13·45 ± 1·1430	13·85 ± 0·1542	13·65 ± 0·1069
+6½	13·35 ± 0·2326	12·45 ± 0·3033	12·90 ± 0'2019	13·60 ± 0·2103	13·98 ± 0·1621	13·85 ± 0·1298
+7	14·30 ± 0·2626	13·00 ± 0·2294	13·65 ± 0·2011	13·45 ± 0·1474	13·70 ± 0·1485	13·58 ± 0·1049
+7½	13·05 ± 0·1859	12·88 ± 0·1901	12·96 ± 0·1325	12·93 ± 0·1804	13·15 ± 0·1318	13·04 ± 0·1117
+8	13·85 ± 0·1542	13·83 ± 0·1822	13·84 ± 0·1210	12·38 ± 0·1463	13·00 ± 0·1008	12·75 ± 0·0892
+8½	13·75 ± 0·2392	12·70 ± 0·2626	12·23 ± 0·1944	12·20 ± 0·2471	12·25 ± 0·1053	12·24 ± 0·0994
+9	13·40 ± 0·1281	12·75 ± 0·2161	13·18 ± 0·1176	13·28 ± 0·1386	13·63 ± 0·1325	13·45 ± 0·0973
+9½	13·30 ± 0·2524	13·55 ± 0·1697	13·43 ± 0·1514	12·65 ± 0·1817	13·35 ± 0·1412	13·12 ± 0·1191
+10	12·45 ± 0·2348	15·13 ± 0·2950	13·21 ± 0·2971	13·35 ± 0·1301	13·90 ± 0·1710	13·72 ± 0·1566
+10½	13·70 ± 0·2065	—	13·70 ± 0·2065	12·60 ± 0·2225	13·35 ± 0·1500	13·10 ± 0·1317
+11	14·00 ± 0·2177	—	14·00 ± 0·2177	13·25 ± 0·1708	13·30 ± 0·1485	13·28 ± 0·1125

A further part of the testis from each labelled individual was used to obtain autoradiographs from Feulgen stained squash preparations (Kodak AR 10 stripping film). After a 30-day exposure period, the autoradiographs were developed in Kodak D19b and fixed in Amfix.

3. RESULTS

(a) *Meiotic timing*

The first wave of labelled cells to arrive at diplotene-diakinesis in experiment A was seen in one of the two irradiated individuals sampled on day 7½.

All injected animals sampled on days 8 and 8½ post-injection from both irradiated and control groups showed label over all meiotic stages from pachytene to anaphase II with the grains being distributed over both homologues. With the single exception of the individual noted above, no other injected animal sampled on or prior to day 7½ had label over any post-zygote stage. In the samples taken on day 9, the only labelled cells were either pre-pachytene or spermatid in nature. Thus, in both irradiated and control groups of experiment A meiosis takes 8 to 8½ days. Of this time prepachytene stages occupy 6-6½ days, premeiotic DNA synthesis up to 1 day and the two meiotic divisions 1 day. Since this is true of both 150 r.-treated and unirradiated individuals, it would appear that the X-ray treatment had no significant delaying effect on meiosis. These meiotic timings were confirmed by analysis of the labelling patterns in experiment B.

TABLE 3

Analyses of variance of the chiasma frequency data

Item	d.f.	S.S.	M.S.	V.R.	P
		(a) *Or v Or+H^3-Thymidine*			
1. Between treatments	1	14·1097	14·1097	<1	>0.20
2. Between times	15	119·6362	7·9757	<1	>0·20
3. Interaction	15	135·6004	9·0400	<1	>0.20
4. Between individuals within times within treatments	19	343·0101	18·0532	20·2731	<0·001***
5. Between cells within individuals	957	852·1649	0·8905	—	—
		(b) *150 r A v 150 r B*			
1. Between treatments	1	33·1941	33·1941	4·9449	0·05*
2. Between times	14	417·9661	29·8547	4·4475	<0·001***
3. Interaction	14	39·7154	2·8368	<1	>0·20
4. Between individuals with times and treatments	26	174·5313	6·7127	8·0585	<0·001***
5. Between cells within individuals	1032	859·6508	0·8330	—	—

(b) *Chiasma scores*

The standard karyotype of *Chorthippus brunneus* has been fully described elsewhere (John and Hewitt, 1966; Southern, 1967). In addition to a single allocyclic X chromosome, the male complement consists of 16 autosomes. On the basis of length and centromere position these can be placed in two distinct groups, one consisting of three pairs of metacentric chromosomes (L_1-L_3), the other consisting of five pairs of telocentric chromosomes (M_4-M_7 and S_8). Because of the practical difficulty of consistently distinguishing between the L_1 and L_2 bivalents and between the M_4 and M_5 bivalents, chiasma frequency values were recorded separately for the six chromosome groups L_{1+2}, L_3, M_{4+5}, M_6, M_7, S_8. The results are summarised in table 2.

Since it was not always possible to score the requisite 20 cells from each individual, the analyses of the data have been carried out in terms of a weighted least squares analysis using KDF9 computer. In each analysis only those times common to both treatments can, of course, be used, since only these contribute to the interaction items.

Two controls, Or and Or + H^3-Thymidine were included in the experimental design to facilitate the detection of any effect on chiasma frequency ascribable to the H^3-Thymidine injection alone. A comparison of these two controls is shown in table 3 (*a*). When tested against the appropriate estimate of error (item 4), none of the three main items of this analysis proves to be significant. Tritiated thymidine alone, therefore, has no detectable effect on mean cell chiasma frequency and so the two controls were pooled. The significance of item 4 when tested against item 5 in the above analysis is to be expected, since this is a measure of the heterogeneity of the genotypes of the individuals used in the experiment.

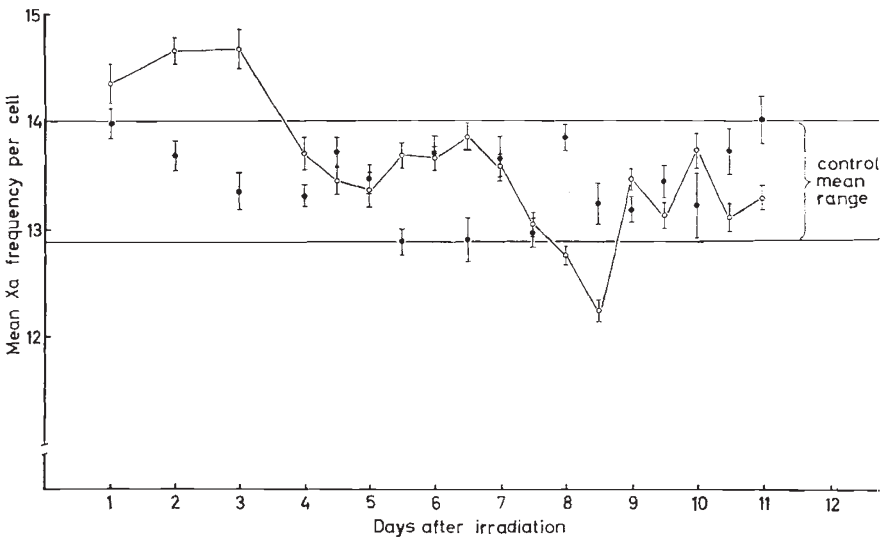

FIG. 1.—Change in chiasma frequency with time. Means and standard deviations are given for irradiated ○ and for control data ●. Overall control mean ± 13.45±0.374.

Table 3 (*b*) shows a comparison of the two irradiated groups of individuals. Although both the between treatments (item 1) and the between times (item 2) main effects appear to be significant, the former is only just so (VR = 4·9449 P = 0·05). It is important to note that the interaction item (item 3 table 3 (*b*)) is not significant. Thus although the chiasma scores in experiment B tend consistently to be slightly higher than in experiment A (see table 2), the absence of any significant interaction item indicates that both groups are behaving in the same way with time. The difference between the mean value for each treatment is probably simply a reflection of the different histories of the batches of insects used in each experiment. Since the patterns of response with time were similar for these two groups, as were the meiotic timings, the two 150 r. treatments were pooled.

The net effect of 150 r. X-rays compared with the control is shown in fig. 1 and table 4. Only the between times item (item 2) is significant when tested against item 4 (VR = 1·8825 P = 0·05-0·01). The results of the analysis shown in this table suggest that not only do the 150 r. treatment and the control not differ from one another in mean cell chiasma frequency but that they both react in the same way with time. This result can probably be

attributed to the large size of item 4 M.S. in the table, since when the control and irradiated groups were analysed separately above, a different conclusion was reached. That the X-ray treatment does indeed affect mean cell chiasma frequency is supported by the results of a series of *t*-tests shown

TABLE 4

Analysis of variance of the pooled control and pooled irradiated data

Item	d.f.	S.S.	M.S.	V.R.	P
1. Between treatments	1	4·8717	4·8717	<1	>0·20
2. Between times	17	315·5779	18·5634	1·8825	0·05-0·01*
3. Interaction	17	235·7575	13·8681	1·4063	0·20-0·10
4. Between individuals within times within treatments	79	779·0262	9·8611	11·6109	<0·001***
5. Between cells within individuals	2140	1817·5807	0·8493	—	—

in table 5. Here the mean of each time of the 150 r. treatment shown in fig. 1 is compared with the overall control mean.

Two main points emerge from fig. 1 and table 5. Firstly there is a significant increase ($P < 0{\cdot}001$) in mean cell chiasma frequency over the control mean on days 1, 2 and 3 post-irradiation, and secondly there is a significant reduction of mean cell chiasma frequency, also at the 0·001 level

TABLE 5

The direction of significant deviations in mean cell chiasma frequency between the irradiated samples and the pooled control sample

Day Post-treatment	*t*	*n*	P	Direction of deviation
1	4·7	1086	≪0·001	+ve
2	8·4	1121	≪0·001	+ve
3	6·6	1090	≪0·001	+ve
4	1·3	1086	0·20-0·10	—
4½	<1·0	1086	>0·30	—
5	<1·0	1116	>0·30	—
5½	1·7	1126	0·10-0·05	—
6	1·5	1126	0·20-0·10	—
6½	2·5	1106	0·02-0·01	+ve
7	<1·0	1126	>0·30	—
7½	3·0	1126	0·01-0·001	−ve
8	5·7	1146	≪0·001	−ve
8½	8·8	1126	≪0·001	−ve
9	<1·0	1126	>0·30	—
9½	2·0	1106	0·05-0·02	—
10	1·7	1106	0·10-0·05	—
10½	2·2	1106	0·05-0·02	—
11	1·2	1126	0·30-0·20	—

on days 8 and 8½ post-treatment. Chiasma scores were made on cells at diplotene-diakinesis and since the most advanced labelled cells on days 1, 2 and 3 were at leptotene-early zygotene, then by extrapolation from the meiotic timings above, these cells showing an increased chiasma frequency were at late zygotene-early pachytene at the time of irradiation. This finding, though not seen in the *S. gregaria* experiment, is none the less in

agreement with the results of Lawrence (1961*a*, *b*) following γ-irradiation of *Lilium longiflorum* and *Tradescantia paludosa*.

The decrease on days 8-8½ coincides with the arrival of labelled cells at diplotene-diakinesis. Thus as in previous experiments, irradiation of cells undergoing premeiotic DNA synthesis leads to a reduction in chiasma frequency. It is interesting to note that the single individual sampled on day 8½ in experiment B which exhibited a more advanced labelling pattern than others taken at the same time also had a higher chiasma frequency

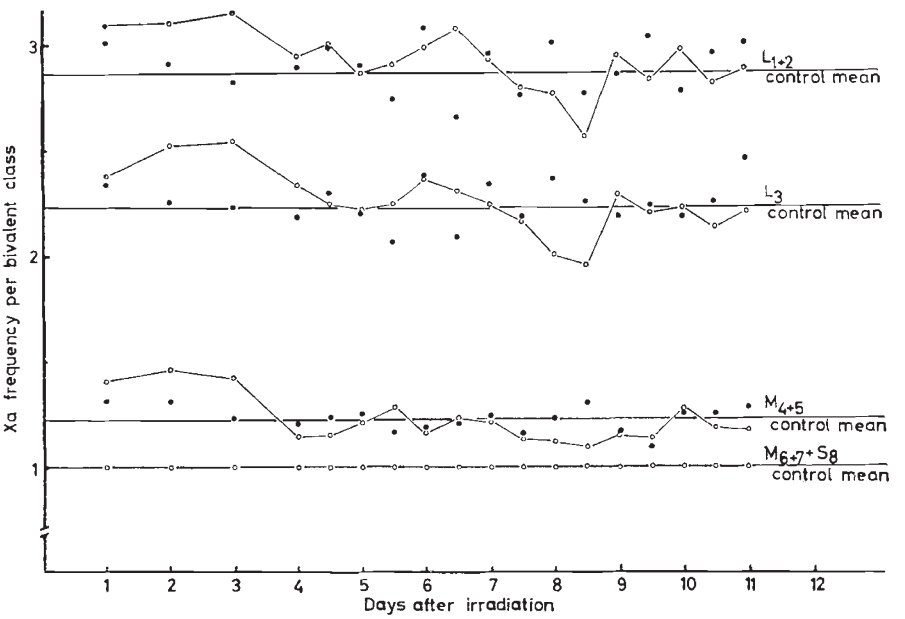

FIG. 2.—The response of the four different classes of bivalent to irradiation (Irradiated data ○—○, control data ●—●).

(12·70; cf. 12·20 and 11·85). Similarly the individual showing labelled diplotene cells on day 7½ in experiment A (150 r.) had a lower chiasma frequency than the other individual sampled at the same time (12·50; cf. 13·35).

Although no really significant increase in chiasma frequency other than that on days 1-3 was recorded, a study of fig. 1 and table 5 does reveal an upward trend about day 6½. Indeed the mean cell chiasma frequency of the irradiated material at this time is significantly greater than the pooled control mean ($0{\cdot}01 < P < 0{\cdot}02$). This increase appears at about the time at which cells irradiated during leptotene-early zygotene would be expected to arrive at diplotene-diakinesis.

The response of the six different bivalent classes scored is shown in fig. 2. Chiasma scores for the three bivalents M_6, M_7 and S_8 have been pooled, since all three continued to form a single chiasma under all conditions. It appears, therefore, that the overall changes in mean cell chiasma frequency are the result of changes in the two groups of metacentric chromosomes L_{1+2} and L_3 and in the telocentric group M_{4+5}. All three groups agree in showing

the increase on days 1 to 3, though the increase above the control level is slightly more marked in the long chromosomes. In a similar way the decrease seen on days $7\frac{1}{2}$-$8\frac{1}{2}$ is much more pronounced in the metacentric groups L_{1+2} and L_3, there being little if any decrease in the M_{4+5} bivalents.

4. Discussion

Several previous studies have indicated the presence of discrete periods in meiosis of a number of organisms at which recombination or chiasma frequency can be altered by both physical and chemical treatments (see table 5, Westerman, *loc. cit.*, for summary). The pattern of response of chiasma frequency to X-irradiation seen in the present experiment was broadly similar to that seen in *S. gregaria* but does differ in a number of ways. Both *Ch. brunneus* and *S. gregaria* agree in showing a significant reduction in chiasma frequency following irradiation of premeiotic DNA synthesis. It is likely that they also agree in showing an increase following irradiation of leptotene-early zygotene, though in *Chorthippus* the increase is small and seen only on day $+6\frac{1}{2}$ ($t_\infty = 2{\cdot}5365$ P = 0·02-0·01). Using another grasshopper species, *Melanoplus femur-rubrum*, Church and Wimber (1969) have also noted an increase in chiasma frequency following irradiation at this time. In their experiment the increase was seen in spite of an overall reduction in chiasma frequency brought about by a 42° C. temperature regime. The most likely target of treatments given at this time in meiosis is the pairing of homologues. This suggestion is strongly supported by the work of Moens (1970). In an elegant experiment Moens has shown that in *Locusta migratoria* the pairing of homologues as marked by formation of synaptinemal complexes takes place some 24-48 hours after the completion of premeiotic DNA synthesis.

The patterns of response differ, however, in that *Ch. brunneus* shows a marked increase in chiasma frequency following irradiation of zygotene-early pachytene—the classical time of chiasma formation. Similar increases in chiasma frequency following treatments given at this time in meiosis have been noted by Lawrence (*loc. cit.*) and Craig Cameron (1970).

As with the desert locust, the effect of the X-irradiation appears to be restricted to the longer chromosomes of the complement irrespective of whether these are metacentric or telocentric in organisation. Indeed, the increase or decrease is most pronounced in the longer chromosomes. Thus, following treatment with 150 r. X-rays, all of the longer chromosomes are capable of forming more chiasmata, though any decrease is to a minimum of one chiasma per bivalent. Under the experimental conditions used, once this minimum is reached the bivalent continues to form its single chiasma. This finding is in marked contrast to those of Henderson (1966) and Peacock (1968). Following heat shocks of 40° C. and 37° C. given to *S. gregaria* and *Goniaea australasiae* respectively, these two authors showed that the observed reduction in mean cell chiasma frequency was accompanied by an increase in the level of univalence. It is of interest to note that in their heat shock experiment, Church and Wimber (*loc. cit.*) failed to observe univalents in any of the individuals sampled. As a result of this finding, these authors postulated the existence of two quantitatively different types of chiasmata. Such a conclusion is however unnecessary. Since chiasma formation at

zygotene-early pachytene is the end-product of an interrelated series of events, each known to be under both major and minor genetic control (Rees, 1961), then the different results may be simply a reflection of different agents affecting these control mechanisms differentially. Thus X-rays and Actinomycin-D might influence chiasma frequency by affecting the " fine " controls of chiasma formation—those governing the distribution of extra chiasmata in the cell over and above the one per bivalent necessary for normal meiotic segregation. Heat shock in the experiments of Henderson and Peacock, on the other hand, seems to affect also those processes which control the formation of this primary chiasma probably through a more direct effect on the pairing of homologues. Just as different genes can affect the coarse and fine controls of chiasma formation, so too can external agents, both physical and chemical.

5. Summary

1. The pattern of response of chiasma frequency to X-irradiation was studied in the grasshopper *Chorthippus brunneus*. In order to relate any observed change to the time of induction, cells undergoing DNA synthesis at the time of irradiation were labelled with H^3-Thymidine. Tritiated thymidine alone had no effect on mean cell chiasma frequency.

2. Two distinct radiosensitive periods were found. X-irradiation at the first of these, corresponding with premeiotic DNA synthesis, led to a significant reduction in mean cell chiasma frequency when scored at diplotene-diakinesis. The same treatment when given at the time of zygotene-early pachytene gave a significant increase. A slight increase was also noted following X-irradiation given during leptotene-early zygotene.

3. All alterations in mean cell chiasma frequency were the result of changes in the number of chiasmata formed by the longer chromosomes—both metacentrics and telocentrics. No univalents were observed and three smallest bivalents continued to form a single chiasma at all times.

4. It is suggested that different experimental agencies may affect the control mechanisms of chiasma formation differentially. In some organisms a particular treatment may affect only the " fine " controls of chiasma formation, in others the same treatment may also affect the " coarse " controls.

Acknowledgments.—I am grateful to Dr Gareth Jones for many useful discussions, to Dr Mary Jones for help with the analysis of the data and to Miss Patricia Wilkinson for her technical asisstance.

6. References

CHURCH, K., AND WIMBER, D. E. 1969. Meiosis in the grasshopper: Chiasma frequency after elevated temperature and X-rays. *Can. J. Genet. Cytol.*, *11*, 209-216.

CRAIG-CAMERON, T. A. 1970. Actinomycin-D and chiasma formation in *Schistocerca gregaria* Forskål). *Chromosoma (Berl.)*, *30*, 169-179.

HENDERSON, S. A. 1966. Time of chisma formation in relation to the time of DNA synthesis. *Nature (Lond.)*, *211*, 1043-1047.

JOHN, B., AND HEWITT, G. M. 1966. Karyotype stability and DNA variability in the Acrididae. *Chromosoma (Berl.)*, *20*, 155-172.

LAWRENCE, C. W. 1961*a*. The effect of irradiation on different stages of microsporogenesis on chiasma frequency. *Heredity*, *16*, 83-89.

LAWRENCE, C. W. 1961*b*. The effect of radiation on chiasma formation in *Tradescantia*. *Radiat. Bot.*, *1*, 92-96.

MOENS, P. B. 1970. Premeiotic DNA synthesis and the time of chromosome pairing in *Locusta migratoria*. *Proc. nat. Acad. Sci.* (*Wash.*), *66*, 94-98.

PEACOCK, W. J. 1968. Chiasmata and crossing-over. In *Replication and Recombination of Genetic Material*, pp. 242-252 (ed. W. J. Peacock and R. D. Brock). Australian Academy of Science, Canberra.

REES, H. 1961. Genetic control of chromosome behaviour. *Bot. Rev.*, *27*, 288-318.

SOUTHERN, D. I. 1967. Chiasma distribution in Truxaline grasshoppers. *Chromosome* (*Berl.*), *22*, 164-191.

WESTERMAN, M. 1967. The effect of X-irradiation on male meiosis in *Schistocerca gregaria* (Forskal). I. Chiasma frequency response. *Chromosoma* (Berl.), *22*, 401-416.